

PROCTODONE, AN INSECT DEVELOPMENTAL HORMONE¹

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This paper presents the results of a study of the endocrinology of growth processes associated with the diapause and postdiapause states of the lepidopteran, *Ostrinia nubilalis*. Insect growth and diapause have been studied intensively by many workers, and there is a voluminous body of extant literature on the subject. In view of the profusion of recent reviews (Wigglesworth, 1959; Van der Kloot, 1960, 1961; Gilbert and Schneiderman, 1961; Harvey, 1962), there is no need for a detailed consideration of published literature here, except as necessary to define the conceptual framework within which the study was undertaken.

According to currently accepted concepts of insect growth, the first step in the endocrinological sequence is the production of brain hormone by neurosecretory cells. Brain hormone activates the prothoracic glands, which then secrete the growth and molting hormone, ecdysone. Diapause is generally considered to be caused, primarily, by a failure of the neurosecretory cells to produce brain hormone (or its precursors), although nothing is known of the cause of such failure. The absence of brain hormone results in an arrest of growth and differentiation, and in a suppression of both biosynthetic and bioenergetic metabolism (Williams, 1946; Harvey, 1962). The concept that diapause is caused only by an absence of brain hormone is so widely accepted that some workers have extirpated the brains from insects not in diapause and subsequently used the brainless insects as experimental animals in a state of diapause (Ichikawa and Nishiitsutsuji-Uwo, 1960; Ichikawa and Takahashi, 1959; Kobayashi and Nakasone, 1960).

During the period of diapause, progressive physiological changes occur that eventually lead to resumption of active growth; these unidentified changes constitute *diapause development* (Andrewartha, 1952). The end-point of diapause development is currently considered to be the renewal of brain activity and the reestablishment of normal hormone production (Van der Kloot, 1955; Williams, 1956).

The present study has involved a reconsideration of the physiological nature of diapause and diapause development, and also a re-evaluation of the position of brain hormone in the sequence of developmental events. One of the underlying factors prompting this study was the accumulating evidence of instances of diapause that appear to involve a suppression of endocrine functions other than brain hormone production alone (Fukaya and Mitsuhashi, 1958; Van der Kloot, 1960; de Wilde, 1961; de Wilde and de Boer, 1961; Cloutier *et al.*, 1962; Beck, 1963).

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Another reason underlying our reinvestigation of the identity and sequence of endocrine functions in insect growth phenomena was the inadequacy of the current theory to account satisfactorily for several aspects of insect photoperiodism—diapause induction, diapause development, and seasonal dimorphism. This point may be illustrated, at least in part, by the experimental results obtained in this laboratory in studies on the photoperiodism of the European corn borer, *Ostrinia nubilalis*. Facultative diapause is induced in this species by short-day photoperiods, and occurs in the mature fifth instar larva. Growth rates during the early larval stages are not reduced by rearing the larvae under diapause-inducing short-day photoperiodic schedules, although nearly all of the larvae enter diapause upon attaining larval maturity. This means that the brain-prothoracic gland-corpora allata endocrine system functioned normally through four complete molting cycles, despite the influence of the photoperiod. Unless one makes some complicated assumptions concerning the effect of photoperiod on changing developmental thresholds, it is difficult (by current concepts) to rationalize the delay of the overt photoperiodic response—diapause—until the fifth instar is attained.

This picture is further complicated by the finding that long-day photoperiods experienced early in larval life modify the intensity of diapause in borers subsequently transferred to short-day photoperiods for the completion of larval growth (Beck and Hanec, 1960). Similarly, long-day photoperiods experienced during early diapause exert an eventual effect on the completion of diapause development (Beck and Alexander, 1964). Such hold-over accumulative effects of long-day photoperiods have been reported not only in the European corn borer, but also in the Colorado potato beetle (*Leptinotarsa decemlineata*) (de Wilde, 1958), the pink bollworm (*Pectinophora gossypiella*) (Adkisson *et al.*, 1963), and in the dimorphism of the vetch aphid (*Magoura viciae*) (Lees, 1963). These several effects, plus the finding of Cloutier *et al.* (1962) that the brain of the corn borer is at least potentially active from an endocrinological standpoint during diapause, suggest that the neurosecretory system of the insect brain may be under the control of a second, unidentified physiological system that is responsive to photoperiod (Beck, 1963).

There is some evidence that the sites of photoperiodic reception in insects include the brain itself (Lees, 1960; Shakhbazov, 1961; Harker, 1960). There is also evidence, however, that the photoperiodic sites of reception are not exclusively cephalic (de Wilde, 1958). The unidentified system suggested to influence neurosecretory processes should be sensitive to photoperiod, but need not be either a cephalic or a central nervous system component. If photoperiodic stimuli influence two interacting physiological systems, photoperiodic responses such as diapause and dimorphism might result from the effects of the photoperiodic regime on the phase relationships between them, provided they are rhythmic growth-controlling functions. One of the rhythmic functions would probably be that of neurosecretion. The other would be the postulated process to which neurosecretion is closely linked.

By this admittedly speculative view, diapause would be the result of the physiological rhythms being forced into an out-of-phase relationship through the phase-setting effects of the photoperiod. Much of the study reported below was designed to test this "phase hypothesis" of insect developmental phenomena.

METHODS AND MATERIALS

The methods used for rearing European corn borer larvae and for inducing either diapause or uninterrupted development were described in an earlier paper (Beck, 1962). The photoperiodic and chemical treatments utilized to accelerate diapause development have also been described in detail (McLeod and Beck, 1963; Beck and Alexander, 1964). Many of the experiments reported herein involved the use of ligation and tissue implant techniques. In general, the procedures used were similar to those employed by many other workers, and warrant no detailed description. In so far as practicable, aseptic surgical techniques were employed.

In most of the experiments, the measured end-point of diapause development was taken as pupation. It should, however, be understood that pupation is actually a postdiapause event. The occurrence of pupation depends not only upon the completion of diapause development, but also upon the completion of a post-diapause molting cycle. Larvae that had been ligated or severely injured by surgical manipulations were frequently unable to undergo ecdysis to the pupal stage. The formation of pupal cuticle was taken as the primary criterion of pupation in all such cases.

RESULTS

A group of diapausing European corn borer larvae, 22 days of age, was placed in a lighted incubator at 30° C. and a photoperiodic schedule of 16 hours of light and 8 hours of darkness per day (long-day photoperiod). These larvae began to pupate about 20 days later; by 30 days half of them had pupated, and all had attained the pupal stage by the forty-fifth day. Comparable larvae placed at 30° C. under a short-day photoperiod (12 hours of light and 12 hours of darkness per day) did not begin to pupate until after the sixtieth day, and at the time the last larva died, 122 days after the beginning of the experiment, only 33% of the group had attained pupal status. Such experiments have clearly demonstrated that diapause development was rate-controlled through the influence of the environmental photoperiod. Very pronounced acceleration of development was obtained by injecting 400 micrograms of ammonium acetate into each larva before placing it into the long-day incubator. Diapausing larvae so treated began to pupate on the eighth day; 50% had pupated by the twelfth day, and all had pupated prior to the thirtieth day (Beck and Alexander, 1964).

Gross determination of the locations of sites of photoreception was made by placing diapausing borers in partially masked glass tubes and exposing them to different photoperiods. The inside diameter of the tubes used was too small to allow the larvae to turn around, and both the inside and outside surfaces of the tube were masked with black paint. The larvae were oriented within the tubes in such a manner that only a selected part of the insect was under the unpainted portion of the tube. Two portions of the larvae were exposed to the influence of photoperiods: (1) the head only or (2) the abdomen only (abdominal segments 7 to 10). Controls consisted of groups of larvae placed in unpainted tubes, but otherwise treated in the same way as the experimental larvae.

Two types of experiments were run: (1) re-induction of the short-day rate of development in larvae that had been previously exposed to long-day photoperiods

TABLE I
Effect of abdominal ligations on the photoperiodic response of diapausing European corn borer larvae (long-day photoperiod)

Position of ligation	Larvae treated (no.)	Incidence of pupation* (%)
None (control)	40	90
7th abdominal segment	110	10
8th abdominal segment	60	35
9th abdominal segment	30	67

* Per cent pupating within 40 days post-treatment.

for 10 days; and (2) long-day acceleration of diapause development in larvae not previously exposed to long-day photoperiods. The experimental results showed that exposure of either the head or the abdomen to the incident photoperiod produced a partial response, as compared to the response of the control group, in which both head and abdomen were exposed. The responses were considered partial in the sense that the rate of diapause development adjusted to the photoperiod less rapidly among the partly masked larvae than among the controls. This effect was interpreted as an indication that extra-cephalic photoreception occurs in the corn borer, with the receptor system probably being located in the abdomen. Our results are in good agreement with those of de Wilde (1958), who found extra-cephalic photoperiodic sensitivity in adult Colorado potato beetles.

The possible existence of a photoperiodically sensitive abdominal system was investigated through a series of ligation and surgical experiments. Diapause development was effectively prevented in larvae that had been bisected by tightly applied ligatures (Table I). Ligations were made sufficiently tight to insure destruction of nervous as well as circulatory communication between the divided portions of the insect (surgical removal of the parts posterior to the ligation did not alter the responses obtained). Ligations posterior to the seventh abdominal segment were progressively less effective. Diapause development is apparently

TABLE II
Determination of the critical period for abdominal system participation in diapause development in European corn borer larvae (long-day photoperiod and ammonium acetate treatment on day 0)

Post-treatment (days)	Post-operation survival (no.)	Pupation within 20 days (%)
0	37	3
1	33	21
2	24	21
3	15	53
4	16	50
5	30	30*

* Pupation incidence from the fifth day on was low because the molting cycle was well advanced in many of the larvae, and abdominal ligations inflicted severe mechanical injury from which the larvae were unable to recover completely.

TABLE III

*Time table for diapause development and pupation in the European corn borer
(long-day photoperiod and 400 µg. ammonium acetate per larva)*

Post-treatment (days)	Abdominal system	Brain system	Prothoracic glands
0	+*	+	+
1	+	+	+
2	±	+	+
3	—	+	+
4	—	+	+
5	—	±	+
6	—	—	±
7	—	—	—
8	----- pupation begins -----		

* + = required; — = not required.

dependent upon functions occurring in the abdomen, principally the seventh and eighth abdominal segments. Since diapause development is thought to lead to the activation of the known endocrine organs, all of which are located in the head and prothorax, the inhibition of diapause development by seventh abdominal segment ligation indicated that the effect of photoperiod must, in part, be exerted *via* an unidentified abdominal system.

The time relationships involved in the participation of the abdominal system in diapause development were determined (Table II). In these experiments, borer larvae in diapause were treated with 400 µg. of ammonium acetate and transferred to a long-day photoperiod. At daily intervals, groups of larvae were ligated at the seventh abdominal segment and then returned to the long-day incubator. The larvae were then observed for pupation occurring within 20 days from the beginning of the experiment. Under these conditions, the per cent pupating rose sharply among larvae ligated after the second day. From the third day on, posterior ligation did not prevent pupation. The "critical period" for abdominal system participation in diapause development was, therefore, between two and three days under these experimental conditions. When tested under long-day photoperiods but without ammonium ions, the critical period was found to be about 12 days. Treatment with ammonium acetate tended to reduce individual variation within the experimental groups, and therefore permitted greater precision in the determination of the developmental sequence.

Ligations at the seventh abdominal segment, made during the first 5 days of the experiment discussed above (Table II), resulted in pupation of only the portion of the larvae anterior to the ligature. However, larvae that were ligated on the sixth and later days developed pupal cuticle posterior as well as anterior to the ligature. This response showed that the critical period for ecdysone-dependence of the pupation process under these experimental conditions was approximately 6 days.

Removal of the heads of diapause larvae periodically after ammonium acetate injection and the beginning of long-day exposure demonstrated that the critical period for brain hormone production was about 5 days. The developmental

schedule during diapause development and postdiapause morphogenesis to the pupal stage was concluded to be approximately as shown in Table III.

Role of central nervous system in diapause development

The possibility that the photoperiodic effect on diapause development is mediated by the central nervous system was investigated. Other workers (Lees, 1960; Shakhbazov, 1961) have reported that the neurosecretory cells of the brain are directly sensitive to light stimuli, and Prosser (1934) and Kennedy (1958, 1963) have reported that the sixth abdominal ganglion of the ventral nerve cord in some arthropods is sensitive to photostimuli. These considerations, especially in view of the fact that the terminal ventral ganglion of the borer larva lies in the seventh abdominal segment, led us to test the hypothesis that the neurosecretory cells of the larval brain are subject to secretory control by impulses originating in the terminal ventral ganglion. The experimental data discussed above are inconsistent with the concept that the neurosecretory activity of the brain is suppressed during

TABLE IV
Effects of ventral nerve cord severance on diapause development

Post-operative photoperiod	Larvae used (no.)	Pupation (%)
Long-day	43	35
Short-day	15	7
Dark	15	0

diapause by inhibiting impulses from the posterior ganglion. If such were the case, ligation anterior to the ganglion should have cut off such impulses and released the brain from the inhibited state. Obviously, such was not the case; seventh-segment ligations prevented, rather than promoted, diapause development. The experimental evidence lends better support to the hypothesis that brain-stimulating impulses might be involved in diapause development.

If diapause developmental changes in the neurosecretory system were induced by impulses from the terminal ganglion, severing of the ventral nerve cord immediately anterior to that ganglion should prevent diapause development. This hypothesis was tested experimentally by severing the ventral nerve cords of diapause borer larvae at the sixth abdominal segment. The operation was performed by microcautery, and the operated larvae were held under three different photoperiodic conditions: long-day, short-day, and continuous dark. The post-operative mortality was exceedingly high (65%), but the pupation data (Table IV) clearly showed that cutting the ventral nerve cord did not prevent diapause development, and the photoperiodic response was still manifested. Dissection of each of the pupae obtained confirmed that the nerve cord had been severed in every case. On the basis of these data, it was concluded that diapause development in the European corn borer does not depend on neural communication between the posterior ventral ganglion and the brain.

Earlier work in this laboratory (Cloutier *et al.*, 1962) has shown that the brain of a diapause corn borer implanted into another diapause borer results in the

pupation of the recipient. Such an effect of supposedly incompetent brains was postulated to be the result of surgical alteration of membrane permeability controlling the exchange of nutrient and metabolites between the brain and the blood. In those experiments, the postoperative holding conditions were always short-day photoperiods, because it was thought that such diapause-inducing photoperiods would constitute the most stringent experimental conditions available. In the present study, the "brain barrier" effect was subjected to further experimentation. We were particularly interested in determining whether or not the effects of implanted brains were subject to photoperiodic influence.

Brains (supraesophageal ganglia only) of diapause corn borer larvae were implanted into other diapause larvae of the same age and developmental history. The operated larvae were then held under long-day, short-day, or continuous dark conditions. The effect of photoperiod on diapause development was clearly manifested (Table V, section A). A much higher percentage pupation was

TABLE V

Photoperiod, brain activity, and diapause development in the European corn borer

Section	Implanted brain activity condition	Recipient condition	Post-treatment photoperiod	Operated larvae (no.)	Observed pupation (%)
A	Diapause	Diapause	Long-day	12	92
	Diapause	Diapause	Short-day	18	78
	Diapause	Diapause	Darkness	17	35
B	Diapause	Diapause + 7th seg. ligation	Short-day	20	0
	Diapause	Diapause + 7th seg. ligation	Dark	22	0
C	Nondiapause	Diapause	Short-day	23	100
	Nondiapause	Diapause + 7th seg. ligation	Short-day	12	67

observed under long-day conditions than in the dark, and the short-day photoperiod produced an intermediate response. The response difference between short-day and dark was greater than expected, and will be discussed in a later section. The implanted brains were recovered by dissection after pupation, and it was found that they did not become associated with the nervous system of the host insect, so the effect of photoperiod could not have been mediated by the insect's central nervous system.

When diapause brains were implanted into diapause larvae that had been ligated at the seventh abdominal segment, no diapause development occurred (Table V, section B). It was, therefore, apparent that the abdominal system was functional in larvae receiving implanted brains, and was required for the completion of diapause development.

When fully active, nondiapause larval brains were implanted into diapause larvae, all of the recipients pupated (Table V, section C). Ligation of the recipient larvae at the seventh abdominal segment did not prevent diapause development and pupation when a nondiapause brain was implanted. These were considered to be crucial experiments, and the results allowed us to draw two important conclusions: (1) the diapause brain is not competent, in itself, to induce morphogenesis; and

(2) the abdominal system is required only until the endocrine capability of the brain is fully established. The experimental results also demonstrate that ligation of larvae at the seventh abdominal segment does not so interfere with the mechanics and hydraulics of the molting process that it blocks pupation. However, mechanical interference was previously shown to be of importance only if the molting cycle was well advanced (Table II).

All of the above experiments on the role of the brain, ventral nerve cord, and abdominal system in a diapause development demonstrated that the abdominal system is intimately involved in photoperiodism and diapause development. It is also apparent that the influence of the abdominal system is not communicated to the brain system *via* the central nervous system. The conclusion is drawn that the brain-stimulating factor must be elaborated in the abdominal system and carried by the blood to the brain, where neuroendocrine functions are influenced. The participation of the abdominal system in the process of diapause development must, therefore, be in the form of a hormone.

Endocrine activity of the abdominal system

Two prominent organs are present in the seventh and eighth abdominal segments of the European corn borer larva. One is the terminal ganglion of the ventral nerve cord, and the other is the anterior portion of the hindgut, termed the anterior intestine. These two structures were examined for histological evidence of secretory activity.

The terminal abdominal ganglion represents a fusion of the seventh and eighth primitive abdominal ganglia, and it innervates the terminal segments, muscles, integument, and posterior digestive tract (Snodgrass, 1935). As mentioned above, the abdominal ganglia of crayfish and some other arthropods have been shown to be photosensitive, with the light-induced nerve impulses originating in the ganglion or in the peripheral nerves, rather than in specialized photoreceptors. If a comparable situation prevails in the borer larva, the photo-induced impulses would stimulate local effectors rather than impulse transmission to the brain. The body wall of the borer larva is translucent, and should constitute no serious barrier to light-stimulation of the ganglion or peripheral nerves. A large plexus of peripheral nerves leads into the terminal ganglion from the posterior extremities of the insect; light-induced impulses might originate in some of these nerves. This aspect of the problem is under current investigation.

The possibility of neurosecretory activity in the posterior ganglion was investigated. A pair of neurosecretory cells was found on each lateral aspect of the ganglion. With paraldehyde-fuchsin staining method (Cameron and Steele, 1959), one cell stained purple and the other stained green. But similar pairs of neurosecretory cells were also found on each side of the ventral ganglia lying anterior to the terminal ganglion. Because the hormone involved in diapause development was traced to the body segment of the terminal ganglion, and not to any segment anterior to that ganglion, it was concluded that these neurosecretory cells were not involved in the production of the hormone in question.

Histological examination of the hindgut during early diapause and diapause development disclosed some striking changes in form and staining properties of certain cells during diapause development. These cells are in the epithelium of a

portion of the anterior intestine located a short distance posterior to the bases of the Malpighian tubes, but anterior to the heavily muscled rectal sphincter.

The epithelial cells of most of the hindgut of diapausing larvae are large, contain ovoid nuclei, and possess relatively homogeneous cytoplasm. The epithelial cells of the particular area in question show evidence of secretory activity: the nuclei are multilobate and the cytoplasm contains granular inclusions and numerous small vacuoles. The granular inclusions were found to stain with paraldehyde-fuchsin in a manner similar to neurosecretory granules. It is, of course, recognized that the paraldehyde-fuchsin technique is not a specific cytochemical identification of chemical structure.

There are three lines of evidence supporting the conclusion that these proctodaeal cells produce the abdominal system hormone required to account for the experimental results presented in an earlier section. Because of its source, the name PROCTODONE is proposed for this hormone. The lines of supporting evidence are:

(1) The cytological signs of secretory activity appear at a time long prior to the manifestation of visible changes in the integument that might be indicative of the initiation of the pupal molting cycle. During diapause the lumen of the hindgut is empty, and we have seen no evidence of secretion from the proctodaeal cells into the lumen.

(2) Diapausing larvae that have been treated with ammonium acetate and transferred into long-day conditions for rapid diapause development display a greatly intensified secretory activity in the hindgut cells. The cells present a turbulent appearance, with greatly increased cytoplasmic volume, large vacuoles, numerous granules, and widespread multilobate nuclei. It is quite apparent that proctodone production has been greatly increased under the influence of ammonium ions.

(3) Evidence of a daily secretory cycle has been found. Whether under short-day, long-day, or long-day plus ammonium ions, the cell activity appears to undergo cyclic changes that are obviously regulated by the photoperiod. Under long-day conditions, the purple-staining granular inclusions accumulate during the hours of darkness, forming large dense clumps in the cytoplasm. A few hours after onset of light, the cells were found to be nearly devoid of granular inclusions, although still displaying the other signs of secretory function.

Attempts to accelerate diapause development by the implantation of fragments of active proctodone-producing tissue have not, thus far, been successful. This failure does not prove that the cells in question have no endocrine function, because extirpation and implantation of the tissue requires separation of the cells from their nervous connection. Integrity of innervation from the posterior ventral ganglion may be important to the physiological function. Because of the large size of the proctodone-producing area of the hindgut, we have not been able to implant a very large proportion of the total active tissue. The fragments implanted may have been insufficient to produce a response.²

² Since the submission of this paper, we have found that aqueous extracts of active proctodaeal tissue accelerate diapause development when injected into late diapause borers (see *Science*, 143: 478 (1964)).

DISCUSSION

The details of the role played by proctodone in insect development have yet to be elucidated, and the following discussion is admittedly speculative. Nevertheless, a few characteristics may be inferred from the experimental results now available.

Proctodone's primary target tissue must be the brain, presumably the neurosecretory cells. This interpretation is supported by the data presented in Table V, where it was shown that the implantation of a fully active nondiapause brain obviated the role of the proctodone-producing abdominal system. Most certainly, proctodone is not a diapause hormone, in the sense of an inhibitor of neurosecretory activity. The experimental evidence is consistent only with the concept that proctodone is a developmental hormone in the full sense of the term.

Secretion of proctodone into the hemolymph is apparently on a daily incremental basis, although its effect on the brain system is summated during diapause development. This conclusion is supported by the finding of a secretory cycle in the hindgut epithelium, and by previously published results (Beck and Alexander, 1964) showing that diapause development is not a reversible process. In the earlier study, it was found that the rate of diapause development could be changed by changing photoperiodic conditions, but the amount of diapause development already accomplished by exposure to long-day photoperiods was not lost when the insects were returned to diapause-inducing short-day conditions. The photoperiodically induced diapause development was summated until the developmental threshold was reached, at which point photoperiod and the abdominal system apparently played no further role—diapause development was completed.

Proctodone production and brain sensitivity to proctodone are postulated to be rhythmic processes, both of which are phase-set by photoperiod. The rate of diapause development is low when these two processes are held in an out-of-phase relationship by a short-day photoperiod. A long-day photoperiod resets the rhythms in a development-promoting phase relationship. The data of Table V, section A, and the results of Cloutier *et al.* (1962) can be tentatively explained on the basis of phase relationships between the brain and abdominal systems. Implanted diapause brains were probably released from photoperiodic phase-setting, and they then adopted a free-running circadian rhythm. If the period of the brain rhythm was different from 24 hours, it would be free to drift into phase with the photoperiodically set abdominal rhythm, and diapause development would be accelerated. Diapause brains implanted into diapause borers maintained in continuous darkness were found to be less effective, and this might be explained on the basis that in the dark, both rhythmic systems were free-running with nearly identical periods, and therefore they could only very slowly drift into an entraining phase relationship.

Diapause development in the European corn borer is rate-limited by the phase relationships between the brain and abdominal systems, such that diapause development may be accelerated by factors that increase the amount of proctodone produced per day (ammonium ion effect) or by photoperiodic schedules that establish favorable phase relationships (long-day effect). A combination of these factors produces extremely rapid diapause development.

It seems most probable that proctodone will be found to play an important role in many insect growth phenomena. Although our experimental work has, thus far,

dealt only with the larval diapause of the European corn borer, the published results of other workers, using a variety of insect forms, indicate a number of effects that might be explained by the postulated action of proctodone and a "phase theory" of developmental control.

Williams (1946) reported that diapause pupae of *Hyalophora cecropia* required from 10 to 12 weeks at about 5° C. in order to complete diapause development. In 1956, however, he discovered that the brains of pupae chilled for only 5 weeks would induce morphogenesis when implanted into unchilled pupae. Competence upon implantation occurred much earlier than competence *in situ*. This effect is apparently identical to that reported by Cloutier *et al.* (1962) for the European corn borer, except that photoperiod rather than temperature was involved in the latter case. Since we have traced the effect to proctodone activity in the case of the corn borer, it seems most likely that proctodone is also involved in diapause development of *Hyalophora cecropia*.

Bounhiol (1943, 1945) reported on the effects of variously placed ligatures on pupation and metamorphosis of the commercial silkworm, *Bombyx mori*. He found that an abdominal ligation, performed shortly after the larva had ceased to feed, effectively prevented pupation. At a somewhat later stage, such ligatures did not prevent pupation. Ligation of the Malpighian tubes during the developmental stage at which pupation could be prevented did not prevent the formation of pupal cuticle to some degree, but did prevent adult differentiation (Bounhiol, 1945). Bounhiol interpreted his results in terms of Malpighian tube function and the possible excretion of inhibitory hormones. His results, however, appear to be inconsistent with such an interpretation, but are in accord with the results of the current study and our interpretation that an abdominally produced hormone—proctodone—is involved in insect morphogenesis.

Davey (1962), working with the American cockroach, *Periplaneta americana*, reported that an extract of corpus cardiacum stimulated muscle contractions in the hindgut. The cardiacum factor was thought to act directly upon the epithelial cells of the anterior intestine; these cells, in turn, released an indolalkylamine which stimulated the nerves controlling muscle contractions in the posterior hindgut. Some similarity between Davey's and our results is apparent in attributing hitherto undetected physiological functions to the epithelial cells of a portion of the proctodaeum. It should be noted, however, that the tryptamine compounds found to be active in the cockroach phenomenon did not produce the ammonium ion effect on diapause development in the European corn borer (Beck and Alexander, 1964).

Recently, Johnson (1963) traced neurosecretory axon pathways from the brain to various parts of the insect body, using several species of aphids. A neurosecretory pathway was traced through the ventral nerve cord to the hindgut. Such results suggest the probability of a feedback regulatory relationship between hindgut and neuroendocrine activities.

There are also lines of evidence that indicate possible proctodone participation in both adult and embryonic diapause. The ammonium ion effect was discovered by Hogan (1961), working with an embryonic diapause. The probable existence of noncephalic photoreceptors that play a part in photoperiodism was pointed out by de Wilde (1958), working with an adult diapause. Adult diapause could not be accounted for on the basis of only a suppression of neurosecretory activity (de Wilde and de Boer, 1961).

Diapause does not occur among insects in which the temperature requirements for "diapause development" are similar to the temperature requirements for growth, and which are growing under environmental conditions where photoperiodic influences are either absent or are conducive to uninterrupted growth and morphogenesis. This situation might occur in populations of species displaying facultative diapause, and in species in which diapause does not ordinarily occur. In these cases, the physiological events constituting diapause development must occur concurrently with morphogenic events. Under such "nondiapause conditions," growth and morphogenesis are not interrupted by a phase separation imposed by genetic and environmental factors, such as temperature and photoperiod, and diapause does not occur. But even in such nondiapause cases, the proctodone-neuroendocrine interaction is probably involved in the programming of growth and metamorphosis as normal components of the insect's endocrine functions. These considerations have led us to avoid calling proctodone a "diapause development hormone."

Recent attempts to formulate plausible theories of form determination have emphasized the role of both environmental and humoral factors in the exchange of genetic information, gene penetrance, and gene suppression (Wigglesworth, 1959, 1961; Williams, 1961; de Wilde, 1961). The importance of the known hormones has been stressed, but one of the main weaknesses of current theories has been their inability to account for environmental effects, particularly periodism. The proctodone-producing system and its phase relationships to other endocrine functions may lead to some clarification of some aspects of the polymorphism problem.

The ability of insects to respond to environmental rhythms of photoperiod and thermoperiod in terms of activity rhythms, metabolic rhythms, form determination, and diapause points to their possession of a time-measuring system—a biological clock (Lees, 1960; Beck, 1963). From the experimental results presented in this report, we have good reason to believe that proctodone production is a rhythmic phenomenon and a photoperiodically controlled response in the European corn borer. It seems quite probable that similar relationships will be found in other insect time-measuring systems. If such proves to be the case, meaningful experimental analysis of many developmental problems should be possible.

It is of interest to note that our finding of proctodone does not lead to a contradiction of already well-established knowledge of insect endocrine functions. What is accomplished, however, is the demonstration that proctodone production precedes brain hormone production in the endocrine sequence, and also provides a connecting link between the extrinsic and intrinsic factors controlling growth.

SUMMARY

1. Diapause development in larvae of the European corn borer, *Ostrinia nubilalis*, was shown to be dependent on a physiological factor originating in the seventh and eighth abdominal segments.

2. Under conditions of an experimentally accelerated diapause development schedule (long-day photoperiod plus ammonium acetate treatment), the action of the abdominal system was required for about two days, the brain system for about

five days, and the prothoracic system for about six days, in order to complete diapause and prepupal postdiapause development.

3. The influence of the abdominal system was shown to be transmitted by the blood stream, rather than *via* the ventral nerve cord.

4. The abdominal system was postulated to elaborate and release a hormone, whose physiological action is to stimulate the neuroendocrine system of the brain.

5. Because the abdominal system hormone was found to originate in the epithelial cells of the anterior portion of the hindgut, it was named *proctodone*.

6. Proctodone production was found to be on a 24-hour rhythmic basis, with hormone release occurring during the light hours of the photoperiod.

7. Proctodone is postulated to play a role in nondiapause growth, polymorphism, periodism, and the several forms of diapause.

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